JOURNAL OF INVERTEBRATE PATHOLOGY 39, 267–276 (1982)

The Effect of Re-release of *Oryctes rhinoceros* Baculovirus in the Biological Control of Rhinoceros Beetles in Western Samoa[1]

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Received January 23, 1980; accepted September 8, 1981

The Rhinoceros Beetle Project in Western Samoa has developed and successfully applied biological methods to control the rhinoceros beetle, a serious pest of coconut palms, by using two specific pathogens, a baculovirus (Family Baculoviridae), and an entomopathogenic fungus, *Metarhizium anisopliae*. The application of virus particularly has markedly suppressed the beetle population and helped revive the copra industry. The virus disease had established itself in the wild beetle population several years after its introduction at a level between 30 and 50%. At the same time an increase in beetle numbers and damage to palm trees was experienced. Therefore, a continuous release of virus into beetle-infested areas was proposed. It was argued that, considering the relatively high level of "natural" virus incidence, further releases of virus into the population would be futile. In a combined research and control program, virus was again re-released into the wild beetle population which was already virus infected. The results show that through re-release the virus level can be raised and the number of beetles and consequently the damage can be reduced. The techniques of the control methods are described. The virus release is very easy and cheap; it requires no chemicals, no special equipment, and it is particularly recommended in situations where breeding places are inaccessible or other methods such as plantation sanitation are either impossible or economically impractical. Above all, the methods are absolutely safe from the standpoint of environmental protection.

KEY WORDS: Baculovirus; biological control of rhinoceros beetles; coconut pests; *Oryctes rhinoceros;* re-release of virus; viral control agents.

INTRODUCTION

The rhinoceros beetle (*Oryctes rhinoceros*, Scarabaeidae) is the most serious pest of the coconut palm in the South Pacific. It was inadvertently introduced into Samoa from Sri Lanka in 1909 and from here it spread to many other islands in the region.

In the biological control against the rhinoceros beetle, the Rhinoceros Beetle Project in Western Samoa has successfully used two pathogens, a fungus, *Metarhizium anisopliae*, and a baculovirus of *Oryctes* (Family Baculoviridae: formerly *Rhabdionvirus oryctes*). The fungus was already present, whereas the virus has been newly introduced.

The virus had been discovered in Malaysia in 1963 (Huger, 1966). The disease is highly specific and only the genus *Oryctes* and a few closely related genera are susceptible. It affects and kills the larvae as well as pupae and adult beetles. It is usually transmitted orally. The virus multiplies in the nuclei of the cells and thus the insects can be utilized to mass produce virus. A particular feature of the disease is the very heavy tumor-like growth (hyperplasia) of virus-filled cells in the midgut of the adult beetle. This growth originates from virus-infected regenerative crypts and eventually fills the whole midgut with virus-filled cell nuclei. A gut filled with virus appears macroscopically as if it were filled with pus (Marschall, unpubl.). Since in the early stage the disease exclusively infects the

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0022-2011/82/030267-10$01.00/0

midgut without involving other organs, the beetles remain capable of flying and mating, although they produce plenty of virus in their midgut and spread it through their feces wherever they fly. This enables us to use them to propagate the disease in the field in an uncomplicated way.

In 1967, the virus disease was introduced into the field in Western Samoa for the first time. This was done by putting macerated *Oryctes* grubs which had been inoculated with the virus, into heaps of rotten coconut wood (Marschall, 1970). This introductory trial was to give an answer to three questions: (1) Is it possible to infect wild beetles easily? (2) Does the virus spread through the beetle population to distant and isolated breeding and feeding areas? (3) Does the virus reduce the beetle population and consequently the damage? The results were clearly positive on all three points. Although the application was still on a trial basis, the disease had not only spread from the three locations of release and established itself over the whole area of both islands of Western Samoa, but had already exercised considerable control. In fact, it had brought about the first tangible reduction in damage and a revival of the copra industry. The most important benefit of the introduction of the virus, however, was the fact that for the first time replanting of coconut palm trees on a large scale could be done again, something which had been virtually impossible since the beetle landed in Samoa.

Because of these positive results, the virus was introduced with equal success to the Tokelaus (Marschall, unpubl.), Fiji (Satya Singh, unpubl.), Tonga (Young, unpubl.), Mauritius, the Seychelles, and recently into the Solomons and Papua-New Guinea.

The first drastic reduction in the numbers of beetles and larvae, as well as in damage, was noticed from 1969 to 1970, after the initial epizootic had swept through the islands. From 1970 to 1975, however, practically no more virus was released in the field in Western Samoa. After 1970, a decrease in virus incidence was noticed and again beetles were found in increasing numbers. During the same time also the damage to palm trees increased. A sharp increase in damage was observed in several outbreak areas where the clearing of bush or felling of large numbers of coconut trees had provided ample breeding facilities. From field collections of beetles and grubs (mostly from trapping) it appeared that a "natural" equilibrium between the virus level and the beetle population had established itself. The level was around 35 to 50% in trapped beetles and 8 to 10% in field-collected larvae. We have no indication that a rise in the beetle population is followed by a rise in virus level; experience seems to point rather in the opposite direction.

From these observations it was inferred that the level at which the virus disease had established itself under natural conditions was not sufficient to control the rhinoceros beetle, and that more virus had to be introduced artificially into the wild beetle population. As a result, when the Samoan–German Rhinoceros Beetle Project commenced in 1975, a program of virus re-release was undertaken. The objective of this program was to control the rhinoceros beetle in some of the worst outbreak areas, but it was also to furnish information on the effect of a re-release of virus into a wild beetle population already virus infected.

MATERIALS AND METHODS

For the combined control and research program, six coconut plantations with moderate to heavy beetle damage were selected. No specific test-area dimensions were set, as coconut groves in Western Samoa are more or less contiguous, and the beetles fly over long distances.

For the monitoring of the virus in the field, the beetles were trapped or collected from crowns. A beetle trap consists of a tin 15 cm in diameter and 10 cm in height over which is placed a slice of coconut wood about 20 cm in diameter and 4–5 cm thick,

cut from a trunk, with a hole 2.5 cm wide in the center. A specific beetle attractant, ethylchrysanthemumate (Rhinolure), is dropped on the underside of the wooden slice. The beetles of both sexes are attracted by the scent, crawl through the hole, and fall into the tin from which they cannot escape. The whole contraption is mounted on a wooden board and attached with wire to the trunk of a coconut palm. The traps were placed at the edge of the coconut plantations at intervals of approximately 50 m. They were checked on one certain day every week, the beetles were removed, and fresh attractant was applied at the same time.

For crown collections, the beetles were picked out from between the emerging fronds or poked out of their tunnels with a wire hook. This procedure could only be done in palm trees 5–10 years old.

The collected beetles were put individually in glass tubes, taken to the laboratory, and microscopical preparations were made 1 or 2 days later. The beetles were dissected, a portion of the midgut epithelium was squashed on a slide, and a smear prepared, which was stained and observed under the microscope. The presence of virus infection reveals itself by the occurrence of the so-called ring zone in the nucleus (Fig. 1). For virus release, the beetles were inoculated orally with a virus suspension of a dosage of 10^{-4} g of virus-infected midgut substance per beetle. The virus was obtained from heavily virus-infected midguts of beetles which consist of almost pure virus with cell fragments. The guts were weighed and then macerated in a tissue grinder with a calibrated amount of sugar water. This suspension was dropped with a calibrated pipette onto the mouths of the beetles which readily sucked it up. The infected beetles were marked with a file on their elytra. They were put in boxes suspended from trees in the plantations from where they flew away at night. Controls for all releases were checked in the laboratory for virus infection. The number of beetles and dates of release are indicated in the figures.

Before each virus release, the beetle population was monitored for 3–6 months in four areas. A survey of beetle damage to palms was conducted (percentage of trees with the first five or six emerging fronds cut). Damage surveys were repeated every 6–9 months. In four plantations, beetles were only trapped: Vaipapa, 200 traps; Puipa'a, 90 traps, on Upolu; on Manono Island 100–200 traps; Lalomalava 150 traps, on Savai'i; In one area they were trapped and collected from crowns: Tausani, 140 traps, on Upolu; and in one area they were only collected from crowns: Afia, on Upolu, 200 palm trees were checked in each crown check. Damage surveys were conducted in Vaipapa, Puipa'a, Lalomalava,[3] and Tausani.

RESULTS

The results of the trap catches are presented in Figures 2–7.

To all appearances, the curves of the trap catches show no trend in any direction but seem to follow an irregular pattern.

In all areas there is a peak in beetle numbers each year during the drier season from May to August, and a low in numbers during the rainy season from December to April. This does not represent a fluctuation in the beetle population but rather indicates that the beetles are reluctant to fly to the traps in bad weather. The difference in catch numbers between the two seasons amounts to about 30% in Puipa'a and about 50% in the other areas.

In Manono (Fig. 4), a sharp drop from a peak in June 1977 is noticeable after a virus release, but the beetle population rises again after a year. In Vaipapa (Fig. 2) we observe the same fluctuation, but the curves show a correlation between low catch figures and high virus incidence and vice versa. Only the beetle population in Lalomalava (Fig. 5) shows a tendency to-

[3] The damage survey at Lalomalava was conducted by Mr. Ulf Beichle.

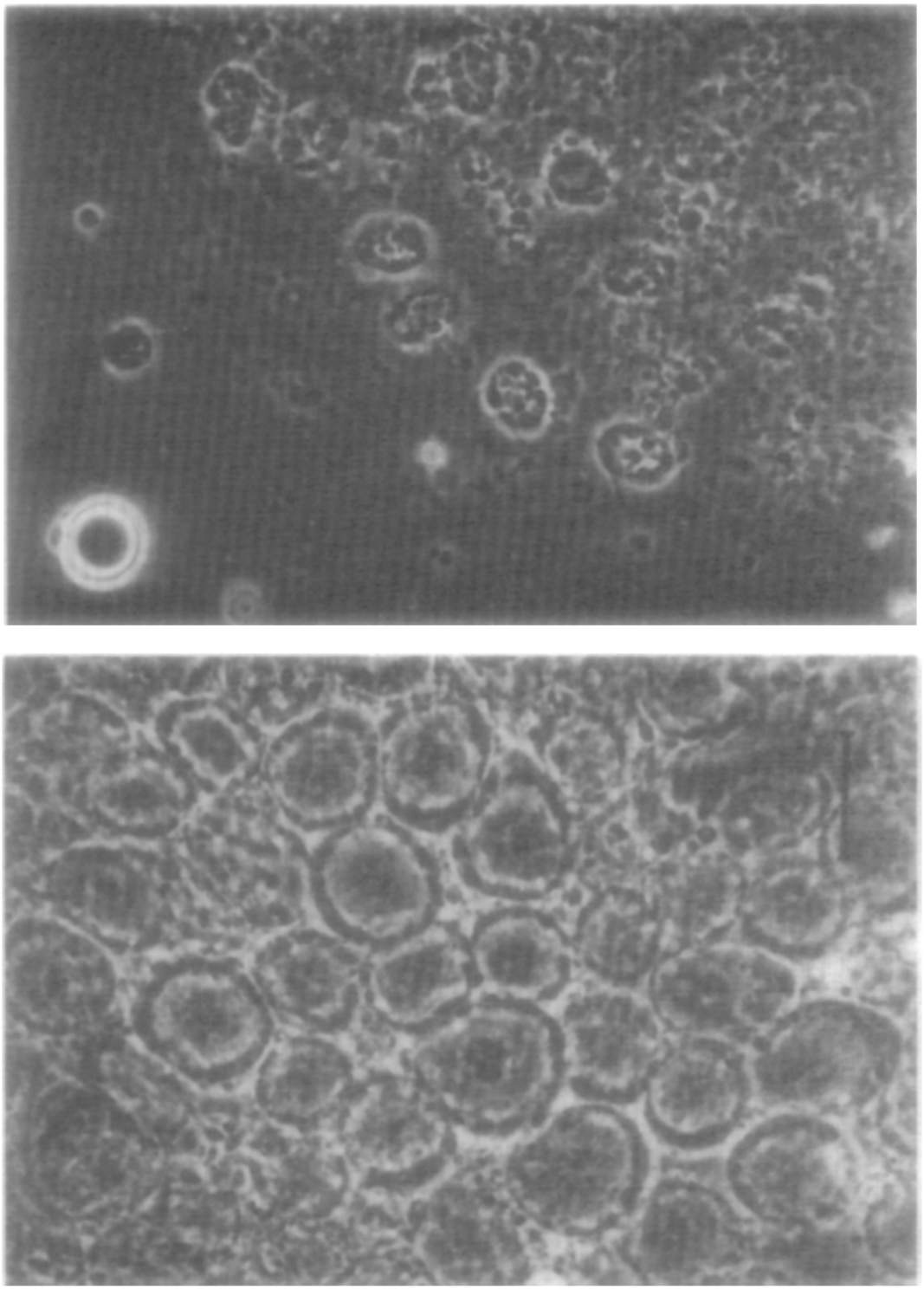

FIG. 1. Healthy and virus-infected nuclei from midgut epithelium of adult rhinoceros beetles. Top: Uninfected nuclei. Bottom: Typical virus-infected nuclei exhibiting the "ring zone." The appearance of the ring zone allows diagnosing the virus infection by means of light microscopy. Midgut epithelium squashed on the slide, fresh mount, phase contrast. Bar = 10 μm.

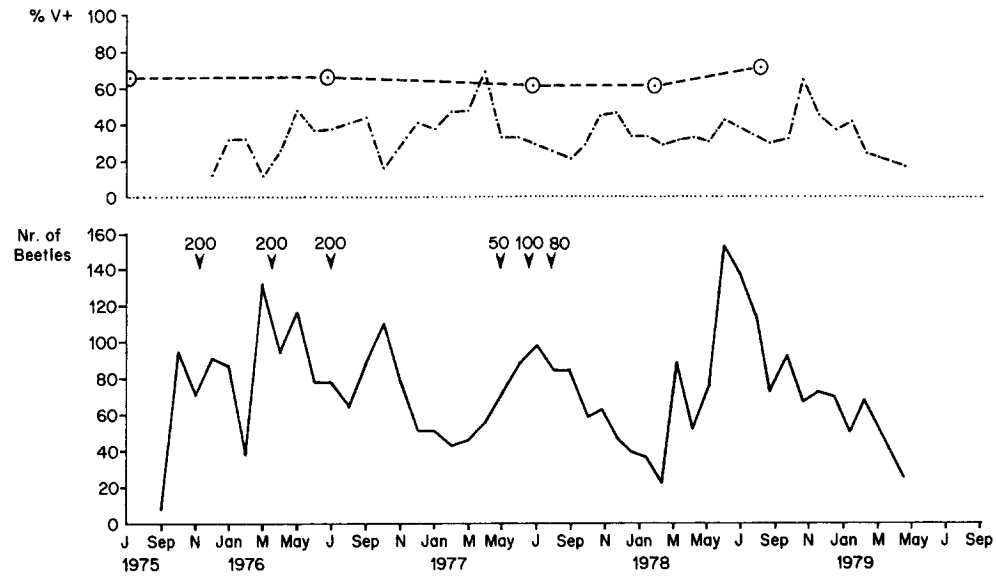

FIG. 2. Graph of trap catches in Vaipapa. Lower curve: Number of beetles caught in traps and virus-infected beetles released in the area. Four catches over a period of 4 weeks are lumped together. Upper curves: percentage of virus infection in the trapped beetles and percentage of beetle-damaged palm trees. Explanation of symbols, Figs. 2–7: 120 ▼, release of virus-infected beetles (numbers refer to number of beetles released); ⊙, percentage of beetle-damaged palm trees; ——, No. of trapped beetles; · – · – · – ·, percentage virus infection.

ward a decline after the application of virus. This is accompanied by a decline in damage as well.

The relative virus incidence (Figs. 2–6, 8) shows even less of a tendency and bears no relationship to the releases or to the seasons. The level of the disease moves irregularly up and down in the range between 30 and 50%, except for a few extremely high or low figures. In Lalomalava, the most successful trial area, judging from the reduction in damage, the level of virus incidence among trapped beetles even drops.

The damage to palm trees in Vaipapa (Fig. 2) first drops slightly, then rises again. This is a typical outbreak area where prior to the trial the palm trees had come under sudden heavy attacks by beetles. The virus

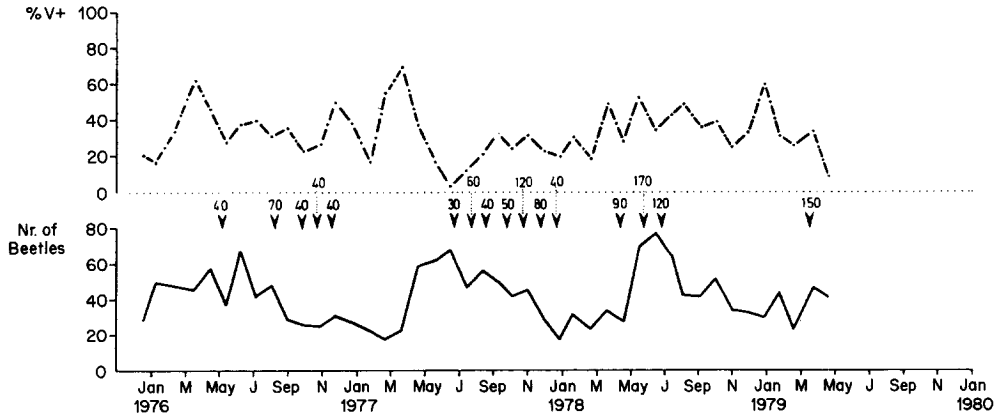

FIG. 3. Graph of trap catches in Puipa'a. Lower curve: No. of beetles caught in traps and virus-infected beetles released in the area. Four catches over a period of 4 weeks are lumped together. Upper curve: percentage of virus infection in the trapped beetles.

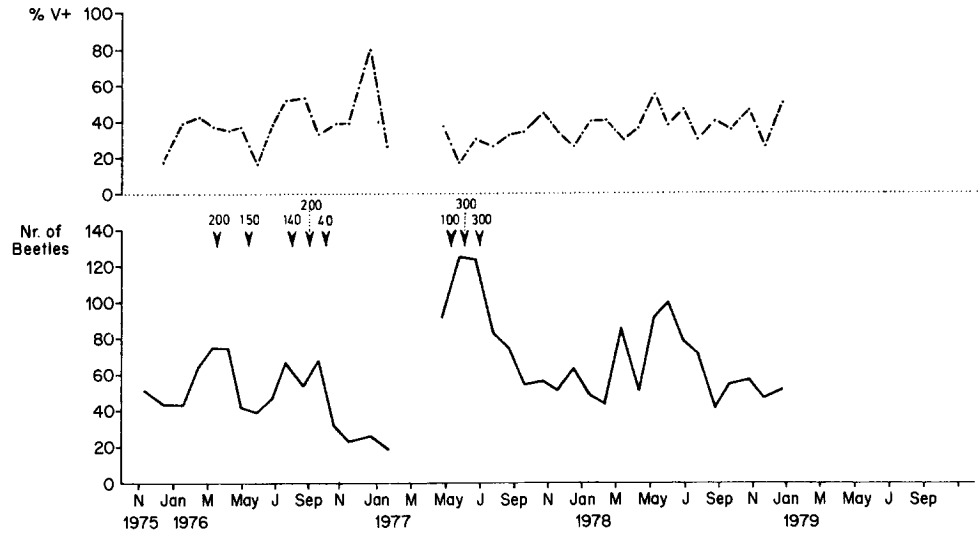

FIG. 4. Graph of trap catches on Manono. Lower curve: No. of beetles caught in traps and virus-infected beetles released in the area. Four catches over a period of 4 weeks lumped together. Upper curve: percentage of virus infection in the trapped beetles.

re-release seemed to have halted these attacks, but when no more virus-infected beetles were released, the damage increased again. In contrast, in Lalomalava (Fig. 5) with continuous virus re-release, the damage shows a steady decline from 75 to 29% over a period of 1 year.

Figure 7 represents the data from crown collections in Afia. In these instances the figures differ essentially from the trapping figures and one notes a clear tendency: right after the re-release of virus the number of beetles collected declines sharply and continues to do so. It drops to 50%

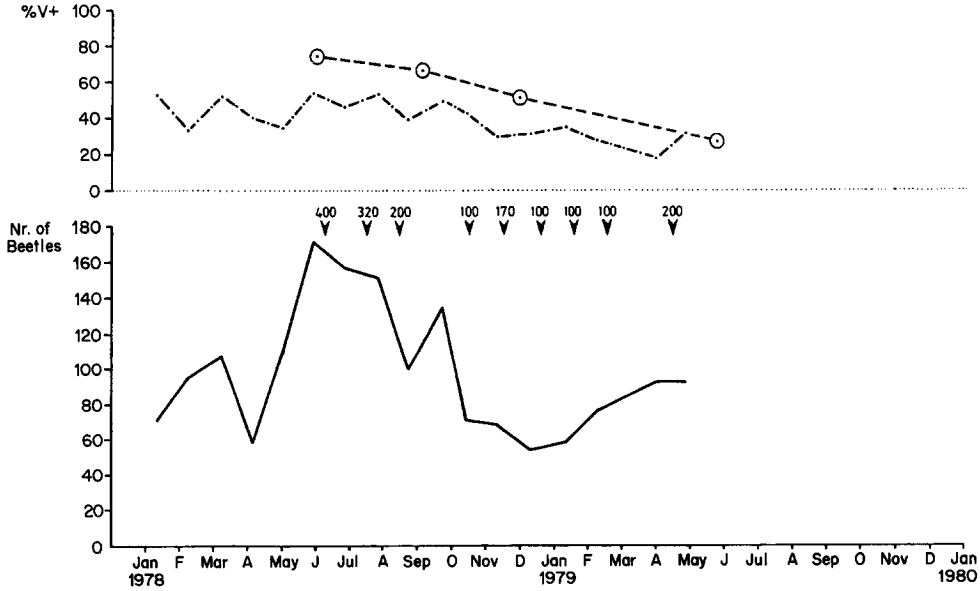

FIG. 5. Graph of trap catches in Lalomalava. Lower curve: No. of beetles caught in traps and virus-infected beetles released in the area. Four catches over a period of 4 weeks lumped together. Upper curves: percentage of virus infection in the trapped beetles and percentage of beetle-damaged palm trees.

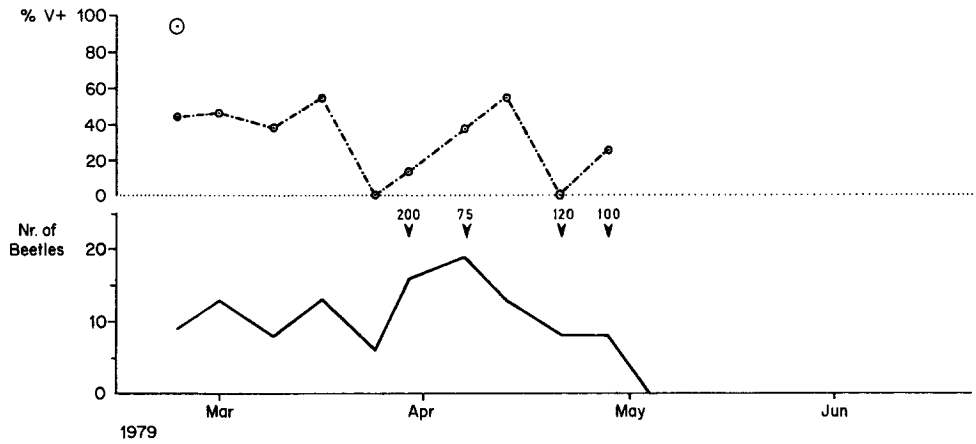

FIG. 6. Graph of trap catches in Tausani. Lower curve: No. of beetles caught in traps and virus-infected beetles released in the area. Weekly catches. Upper curve: percentage of virus infection in the trapped beetles.

after about 2 months. The last collection of beetles, 8 months after the first re-release, yields only less than 5% of what it was before. Unfortunately, in Tausani (Fig. 6, trapping), we caught few beetles to begin with, but the figures follow the same pattern as in the Afia trial.

At the same time the level of the virus disease rises considerably (from 54 to 83% in Afia). It does not go up and down but remains high. We observe a correlation between increasing virus level and decreasing beetle numbers. Even though in the last catch of the Afia trial the virus level drops, that is understandable if one considers the small number of only four beetles from 200 palm trees.

In contrast to the results from crown

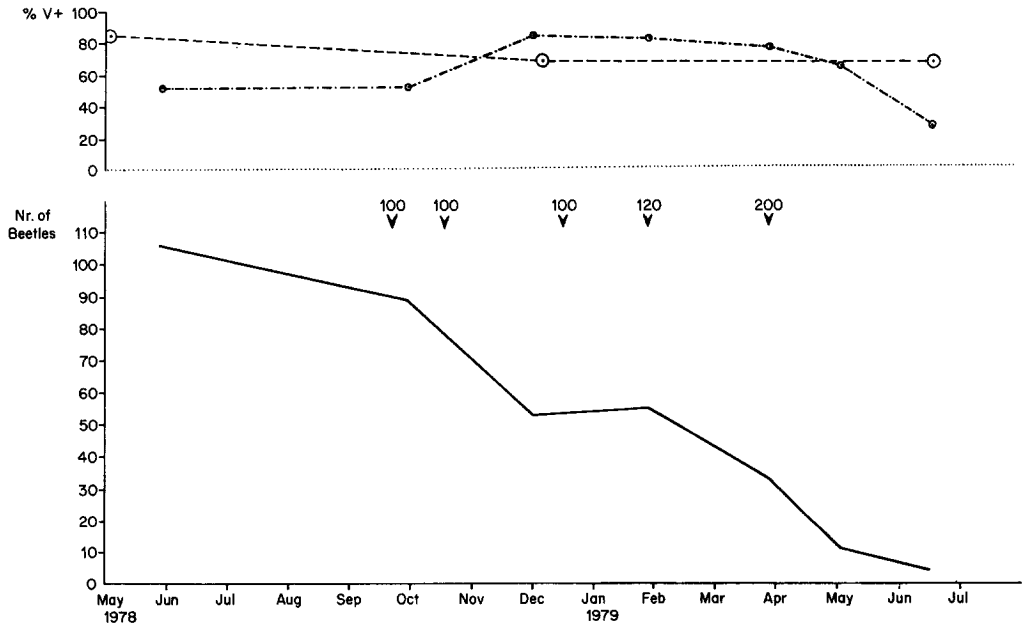

FIG. 7. Graph of crown catches in Afia. Lower curve: No. of beetles collected from crowns and virus-infected beetles released in the area. Upper curves: percentage of virus infection in the collected beetles and percentage of beetle-damaged palm trees. Please note that the curves of the number of crown-collected beetles and of their virus incidence are the only ones showing correlation.

catches, the figures of beetle numbers and virus incidence from trap catches in Tausani (Fig. 6) during the same time follow an irregular pattern as in the other trapping areas.

At the same time, the damage to palm trees decreases slightly. It may be noted that the trees in Afia suffered from continuous heavy attacks by beetles over many years.

In Figure 8 the relative virus incidences in trap catches and crown catches from four areas over the same period are compared. Whereas the trap catches remain in a band between 25 and 50% irrespective of virus release, the crown catches rise after the application of virus and remain high.

DISCUSSION

From the scientific point of view it would have been desirable to have an untreated control area for comparison. However, this was impossible since we had to depend on the plantation owners for support in material and labor and no one could be asked to leave his plantation untreated without financial compensation. However, the area in Vaipapa where no virus beetles were released for more than 1.5 years may be considered a control area.

As for possible repercussions on the beetle numbers due to the taking of beetles from the field for dissection, it is a known fact that trapping or collecting from crowns does not reduce the numbers of the beetle population as a whole to an appreciable degree, even after extensive trapping or collecting. Moreover, beetles usually do not stay more than 3–6 days in one crown but are constantly on the move. This is corroborated by hand-picking of rhinoceros beetles in young oil palm plantations in Malaysia. The method gave a certain protection to the palms but had no noticeable effect on the beetle population. Trapping without other control measures showed in several instances no effect on the beetle numbers. For these reasons the effect of trapping and collecting beetles in the context of the experiments described here can

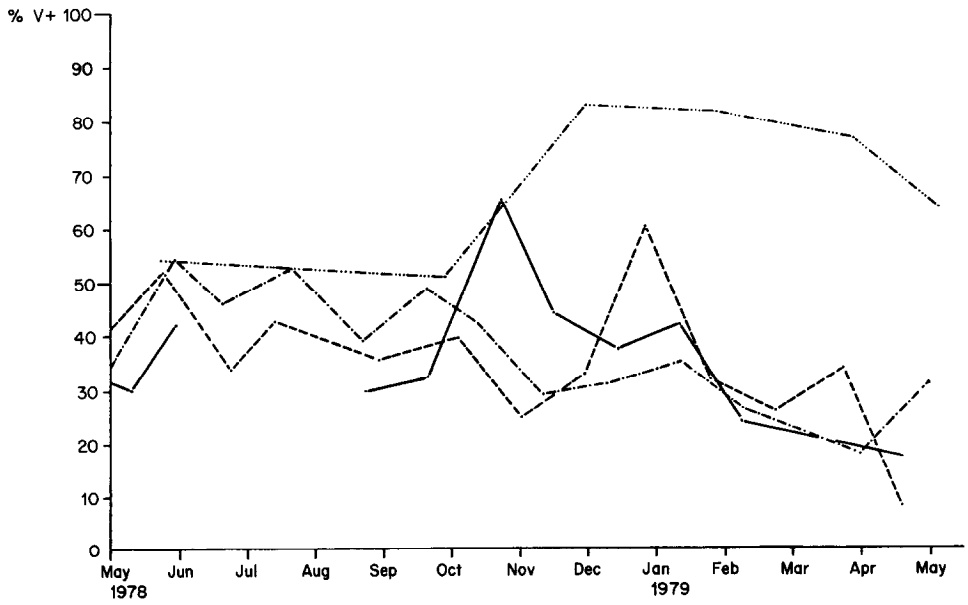

FIG. 8. Graph of relative virus incidence in trap and crown catches. The percentage of virus incidence differs considerably between the beetles caught in traps (the three lower curves) and those collected from crowns (upper curve). Explanation of symbols, Figs. 8 and 9: ——, Vaipapa trap catches; – – –, Puipa'a trap catches; – · – · –, Lalomalava trap catches; – · · · – · · · –, Afia crown catches; – · · · – · · · –, Tausani crown catches; ....., Tausani trap catches.

be neglected. The change in beetle numbers is therefore attributed to the effect of the re-release of virus-infected beetles.

It should be remembered that the beetles, despite their destructive characteristics, occur only in relatively small numbers. A few beetles (about one beetle per three to five palm trees) are capable of inflicting heavy damage. Thirty to forty beetles from 150 traps per week is a very good catch. Also, we have no control over the movements of the beetles once they are released. We know, however, that released beetles first fly to the palm trees and only after having fed they come to the traps (Sabatini, 1979). This explains the very low rate of recapture of released beetles. Sabatini's investigations revealed further that in trap catches there is no bias as to sex, age, or physiological stage of the beetles, i.e., we catch a cross section of the whole population. However, virus-infected beetles are less likely to be caught in traps than healthy ones. So, in regard to the level of the disease, the trap catches are not representative. This explains why the curves of the virus percentages bear no relationship to the releases.

Male beetles are found to have a higher virus incidence than females, about 10–20% more in trap catches. Therefore, if a population is affected by the disease, fewer males than females are caught in traps. This was demonstrated very clearly by Bedford et al. (1975) on the small island of Vomo, Fiji, where they had trapped beetles. Before virus got into the population, the percentage of trapped males was 58.7%; when this changed to 32.2%, virus was discovered in the population.

In Figure 9 the percentages of males from different sources are compared. In the mass rearing (as well as in field collections), we find almost equal numbers of females and males (males 46.4%). If we add up the total of all crown catches, we also find nearly equal numbers of the sexes: in Afia 41.9% males, and in Tausani 44.9% males. The same applies to Vomo trap catches before the virus appeared (58.7% males). Quite in contrast, in virus-infected areas the percentages of males in traps (all catches

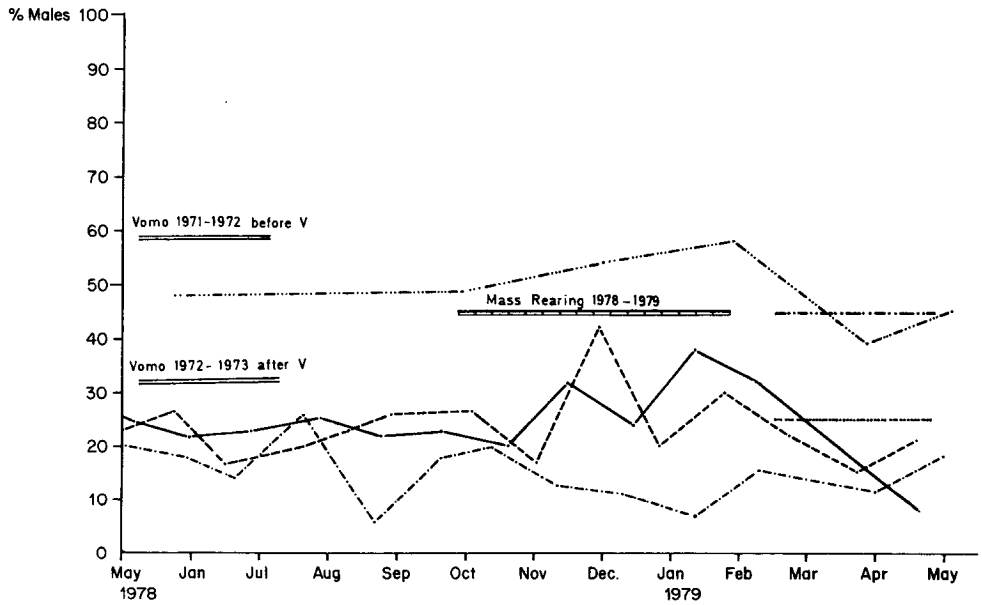

FIG. 9. Graph of relative No. of males in trap and crown catches and from mass rearing. The percentages of males in trap catches from virus-free wild beetle populations, in crown collections, and from mass rearing are all in the range between 40 and 60% and differ markedly from those of trap catches of virus-infected wild populations.

lumped together) range conspicuously lower, namely 32.2% in Vomo, 27.4% in Vaipapa, 24.2% in Puipa'a, 19.5% in Manono, and 17.3% in Lalomalava. In the area with the strongest reduction in damage, Lalomalava, we counted the lowest relative number of males. That suggests that the proportion of males in traps is a better indicator of the disease level and of the effect of the re-release than the virus incidence in trapped beetles.

With males and females being equally frequent in crowns, those figures for virus incidence are more likely to be representative and consequently rise after new application of virus.

From early 1970 to 1975 practically no virus was released in Samoa. It was argued that re-release of virus into an already contaminated wild population would be futile since even the largest possible numbers of infected beetles which could be treated would make but an insignificant addition to the already existing virus level and would therefore be worthless. The results presented here show that the level of virus can be substantially raised and the beetle population reduced by releasing even small numbers of infected beetles. After the re-release of virus into outbreak areas the damage to palm trees decreased.

From those facts the following conclusions are drawn: (1) The virus disease, if left alone at its natural level, does not control the beetle population sufficiently. Experiences from countries with indigenous virus infestation of rhinoceros beetles corroborate this observation. (2) It is possible to increase through re-release of virus-infected beetles the level of virus infection in the wild beetle population and to reduce the number of beetles and subsequently the damage.

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